International Journal of Scientific Research and Engineering Development-– Volume 6 Issue 3, May-June 2023

Available at www.ijsred.com

RESEARCH ARTICLE OPEN ACCESS

Microbiological Safety of Raw and Ready-to Eat Barbecue Meat Sold within Calabar, Cross River State

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ABSTRACT-Meat is one of the major sources of animal protein to humans. Minimum daily protein requirement for an adult is 85.9% with about 34-40kg recommended to be derive from animal origin. The necessity of protein to man cannot be over emphasize. Despiteits importance to human health, researchers found that several foodborne outbreaks arise after the consumption of roadside meat.This may be due to pathogenic organisms that comes in contact with them shortly before consumption. This activated our curiosity to carryout Microbiological evaluation of three; barbecue chicken (BC), Suya meat and freshly slices beef (FSB) different types of meat within our locality (Calabar Metropolis) for public safety. The different meat types were analyzed using Standard Microbiological Techniques.Bacterial mean cell count ranged from 4.6 to 9.5 x 10^8CFU/g. 9.5 x 10^8 CFU/g for Suya meat, 9.0 x 10^8 CFU/g for barbecue chicken (BC) and 4.6-7.3 x 10^8 CFU/g for FSB respectively. 17 bacterial species comprised of G +ve and –ve pathogens were recovered from all meat samples. 69.2, 15.4 and 7.7% were prevalence rate of G-ve rods and cocci. Gram +ve rods and cocci were the leading strains with *Proteus Sp* being the most abundance with prevalence rate of 34.3 % in BC. 45.5, 27.3 %, 18.2, 9.1 % were % values of G-ve and +ve rods, G +ve, -ve cocci with *B. cereus* being the most frequently (25.9 %) isolated organisms from Suya meat. Similar results of Suya meat observed for FSB though higher rate of occurrence. The order of contamination in analyzed meat was; **FSB > Suya meat > BC**. Significance difference was observed at ***P* = 0.5 among Gram negative (G-ve rods)** in all analyzed meat samples compared to Gram positive (G+ve) rods.Bogobiri was the most contaminated location with % contamination rate of 44% to Ekpo Abasi (25 %), Yellow Duke (16 %) and Abang Asang (15 %). Antimicrobial agents used expressed inhibitory potential on some isolates, some were intermediate and resistance. Pathogenic organisms recovered from the findings of the index study reveals the unhealthy status of all experimented meat type. The findings of this research calls for urgent intervention of Government agencies in charge of food safety in the State for quick intervention by providing a bacteriological standard that will serve as a guide to roadside vendors.
This will serve as a better way of minimizing foodborne outbreaks.

Key Words:*Abang Asang. Bogobiri, Ekpo Abasi, Yellow Duke, Pathogenic organisms,barbecue chicken (BC), suya meat, freshly slice beef (FSB)*

I. INTRODUCTION

Meat ranks among one of the most significant, nutritious and preferred food easily access by the general populace.The commonly known types of meat include; red meat (beef, goat, and lamb), poultry (chicken, turkey), pork (pigs), and seafood (fish, crab, lobster).

Theyform the major sources of protein with high biological values ranging from physical / mental development, providesvitamins that serve as an essential growth factor necessary for repairs and maintenance of body cells for human worldwide [1].The minimum required protein daily demand for an adult is 85.9% with about 34-40kg recommended to be derive from animal origin. Research records that Nigeria consumes about 360,000 tonnes of beef each year accounting for half of all West Africa. Aborisade and Carlos. (2017)[2] also has it that there is increase in house hold demand for beef in Nigeria. This exorbitant increase in rate of meat consumption may be linked to it palatability due to different methods of preparation and road side presentation to attract the attention of the road users. Unlearned individuals with no knowledge of food handling dabble into this meat processing as business, source of income to sustain livelihood without formal nutrition training background.

Chukwuma, 2017 [3] reports that 26% constant meat consumption predisposes an individual to infection compare with their counterparts that consumes less. This increases our concern to examine the microbiological status of road sides ready to eat meat types for public safety.

II. Materials and Methods

This study was conducted in Calabar metropolis, Cross River State, Nigeria. The city has an area of 406 square kilometers (157 sq mi) and a population of 371,022 as at the 2006 census. Calabar is the capital of Cross River state. It was originally named Akwa Akpa, in the Efik language. The city is adjacent to the Calabar and Great Kwa rivers and creeks of the Cross River

A. Sample Collection, Preparation, Isolation and Characterization of Bacteria Cell Count

A total of forty (40) samples comprised of twenty barbecue chicken, ten (10) each of freshly slides cow beef and suya meat were randomly purchased from Ekpo Abasi, Yellow Duke, Bogobiri and Abang Asang within April and October, 2021. Each sample were wrapped in aluminum sterile foil paper and placed in a clean polythene bag. Contents were immediately transported to Microbiology laboratory, Cross River University of Technology, CRUTECH, Calabar. A 5g weight of each sample was cut into tiny pieces and homogenized in a sterile blender (LANDERS-YCIA. S.A.) for 2 minutes. Approximately 1g of the homogenate was suspended in 9mL of sterile normal saline for stock solution. 1mL of the suspension was used to perform a ten-fold serial dilution down to 10^{-10}. A 1mL amount of 10^{-8} and 10^{-10} dilution of the meat homogenate suspension was placed in a clean sterile Petri dish; then, 20 mL of molten Nutrient and MacConkey ((Liofilchem® s.r.l., Italy) agar at about 44°C was added and the Petri dish gently swirled until the contents mix thoroughly. The agar was allowed to set before incubation at 37°C for 24 hours in a humidified incubator. After 24 hours of incubation, the plates were examined for growth, morphological appearance of colonies were described, counted and recorded. Thereafter, discrete colonies were isolated, purified after three successive sub-culturing and re-isolations on Nutrient agar. Isolates were characterized by standard bacteriological techniques as described by Cheesbrough (2006) [4].

BData Analysis

The statistical analysis was done using SPSS version 20 for descriptive statistics. The student Unpaired T-test compared the means of BC, Suya meat and FSB that were significant at P = .05.

3.III. RESULTS AND DISCUSSION

3 A. Bacterial Cell Count

Bacterial cell count obtains from BC, FSB and Suya meat ranged from 4.6 to 9.5 x 10^8 CFU/g. 9.5 x 10^8 CFU/g for Suya meat, 9.0 x 10^8 CFU/gfor barbecue chicken (BC) and 4.6-7.3 x 10^8 CFU/g for FSB respectively

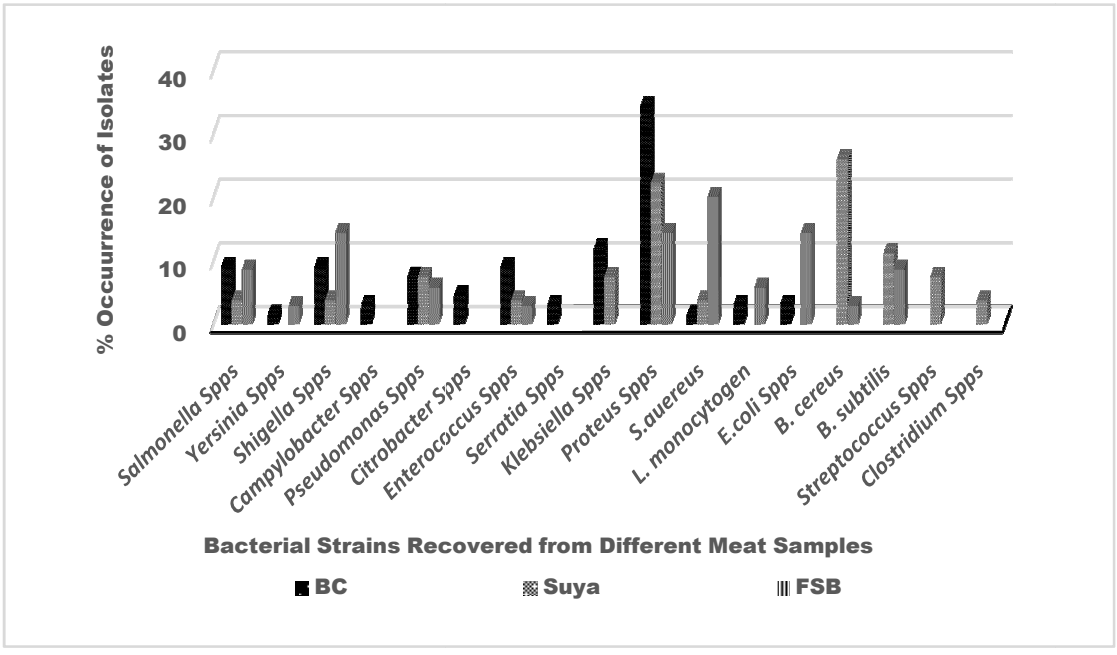

Figure 1: Bacterial Strains Recovered from Different Meat Type

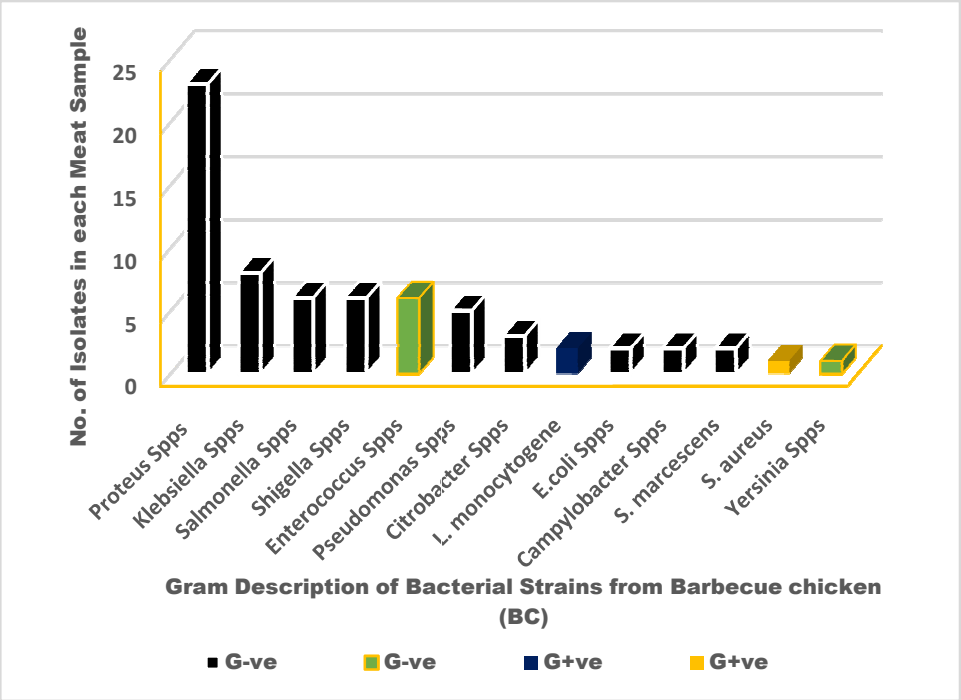

Figure 2:Gram Description of Bacterial Strains from Barbecue chicken (BC)

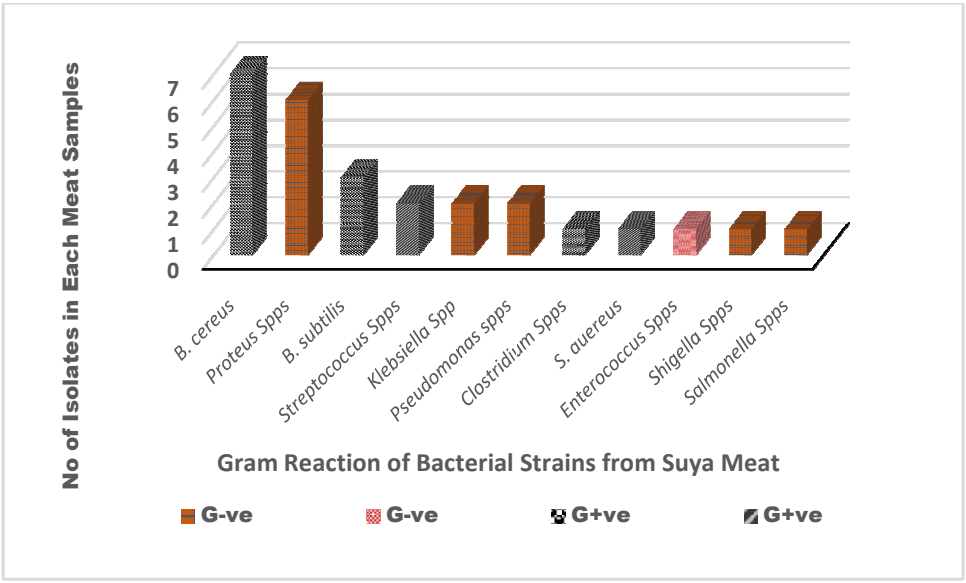

Figure 3:Gram Reaction of Bacterial Strains from Suya Meat

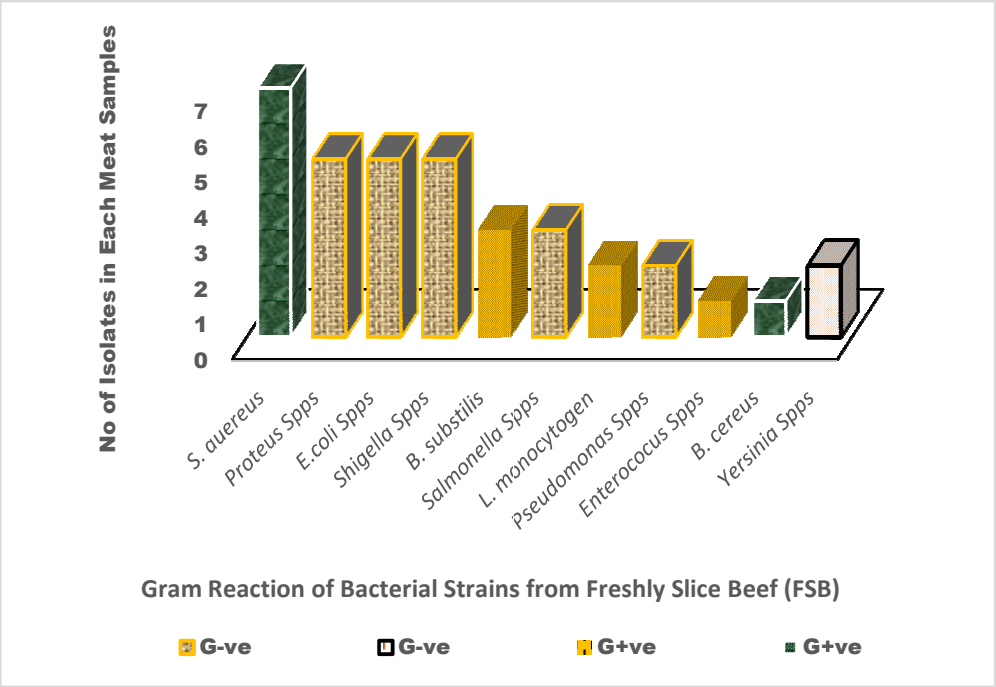

Figure 4:Gram Reaction of Bacterial Strains from Freshly Slice Beef (FSB)

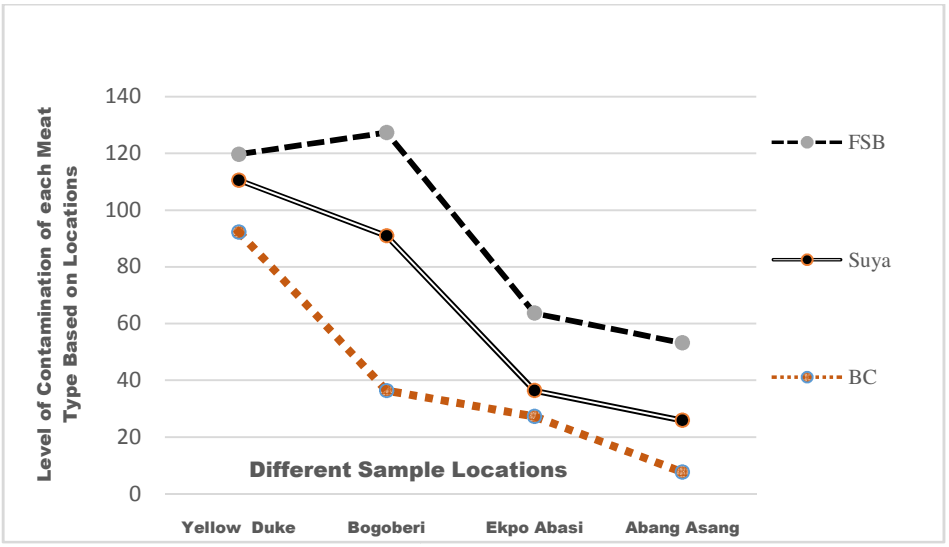

Figure 5: Contamination of Meat Type Based on Sample Locations

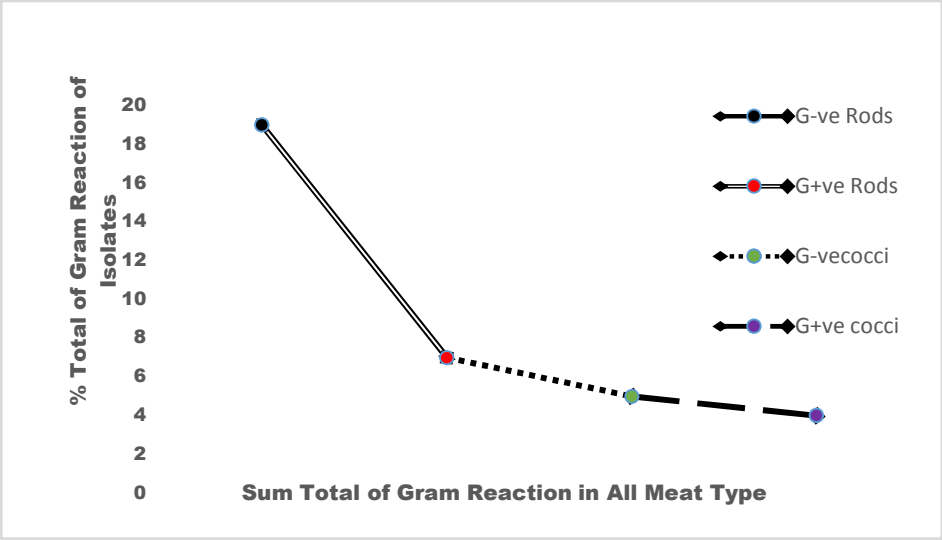

Figure 6: Sum Total of Gram Reaction in All Meat Type

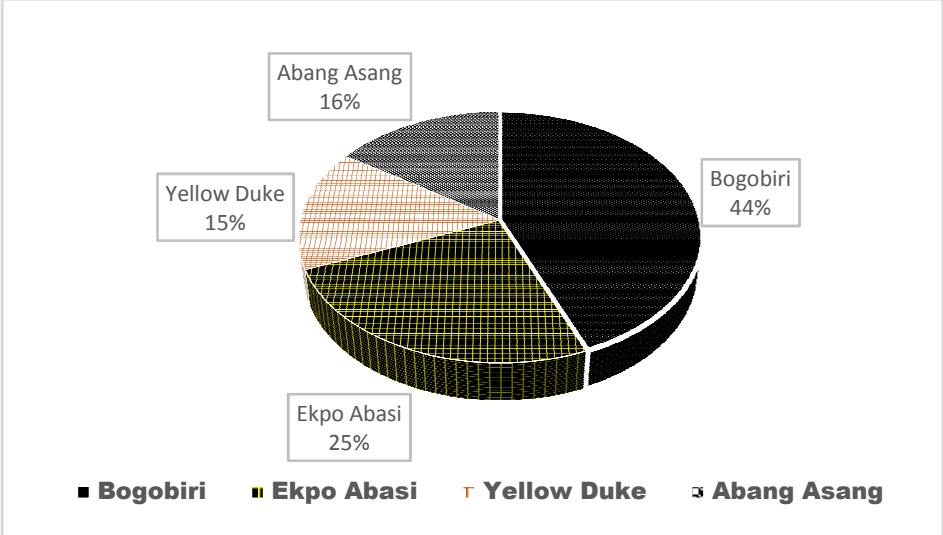

Figure 7: Sum Total of Meat Contamination in All Sample Locations

Table 1: Antibiotic Sensitivity Profiles from Meat Isolates

		Salmonella Sp	*Yersinia Sp*	*Shigella Sp*	*Campylobacter Sp*	*Pseudomonas Sp*	*Enterococcus Sp*	*Proteus Sp*	*S. aureus*	*B. cereus*	*B. subtilis*	*Streptococcus Sp*	*Clostridium Sp*
		Inhibition Zone of Diameter (mm)											
Antimicrobial agent	**Conc. (µg)**												
Perfloxacin (PEF)	10(µg)	28 (S)	32 (S)	28 (S)	00 (R)	28 (S)	26 (I)	28 (S)	36 (S)	34 (S)	32 (S)	32 (S)	28 (S)
Gentamycin (CN)	10(µg)	26 (S)	24 (I)	26 (S)	00 (R)	30 (S)	14(R)	24 (I)	26 (S)	22(I)	22 (I)	22 (I)	18 (R)
Ampiclox (APX)	30(µg)	00 (R)	00 (R)	00 (R)	00 (R)	00 (R)	00 (R)	00 (R)	22 (I)	00 (R)	00 (R)	10 (R)	22 (I)
Zimacef (Z)	20(µg)	00 (R)	00 (R)	00 (R)	00 (R)	00 (R)	20 (I)	00 (R)	28 (S)	22(I)	24 (S)	28 (S)	24 (S)
Amoxillin (AM)	30(µg)	00 (R)	20 9R)	00 (R)	00 (R)	20 (I)	00 (R)	00 (R)	24 (I)	00 (R)	00 (R)	18 (R)	20 (I)
Rocephin (R)	25(µg)	00 (R)	00 (R)	00 (R)	00 (R)	00 (R)	22 (I)	00 (R)	24 (I)	30 (S)	22 (I)	28 (S)	28 (S)
Ciprofloxacin (CPX)	10(µg)	26 (S)	30 (S)	26 (S)	20 (I)	36 (S)	18 (R)	20 (I)	26 (S)	32(S)	30 (S)	32 (S)	32 (S)
Streptomycin (S)	30(µg)	22 (I)	20 (R)	14 (R)	00 (R)	24(S)	00 (R)	00 (R)	30 (S)	24 (S)	28 (S)	26 (S)	28 (S)
Septrin (SXT)	30(µg)	00 (R)	20 (R)	24 (S)	26 (S)	24(S)	00 (R)	18 (R)	22 (I)	(24(S)	24 (S)	22 (I)	20 (I)
Erythromycin (E)	10(µg)	00 (R)	00 (R)	00 (R)	00 (R)	00 (R)	21 (I)	00 (R)	28 (S)	24 (S)	30 (S)	26 (S)	20 (I)
Chloramphenicol (CH)	30(µg)	12 (R)	30 (S)	12 (R)	30 (S)	24 (S)	00 (R)	18 (R)	00 (R)	00 (R)	00 (R)	00 (R)	00 (R)
Sparfloxacin (SP)	10(µg)	16 (I)	26 (S)	16 (I)	20 (I)	30 (S)	00 (R)	24 (I)	00 (R)	00 (R)	00 (R)	00 (R)	00 (R)
Augmentin (AU)	10(µg)	00 (R)	4 (R)	00 (R)	00 (R)	20 (I)	00 (R)	00 (R)	00 (R)	00 (R)	00 (R)	00 (R)	00 (R)
Tarvid (TV)	10(µg)	28 (S)	00 (R)	28 (S)	00 (R)	30 (S)	00 (R)	10 (R)	00 (R)	00 (R)	00 (R)	00 (R)	00 (R)

Key: S= sensitive, I= intermediate, R=resistance, mm=millimeter, µg= microgram.

Meat serve as a major source of protein with high biological values. Different methods of meat preparations upgrade it palatability and increases it demand both in develop and developing Countries.Microbiological safety of different meat types and antimicrobial activity of some selected chemotherapies on isolated organisms was the major subject of the study.

B. Bacterial Cell Count

Three different meat types (BC, Suya meat, and FSB) from open road side vendors at 4 different locations in Calabar Metropolis reveals 4.6 to 9.5 x 10^8 CFU/g bacterial cell count.

C. Different Bacterial Strains From All Experimented Meat Types

Seventeen (17) bacterial strains comprised of Gram positive and negative pathogens were recovered in this study**(Figure 1).**All analyzed meat samples were contaminated with pathogenic organisms. This result is in close agreement with the report of Isiaka *et al.,* (2014)[5] who isolated various strains of pathogenic organisms including; *S. aureus, E. coli, Pseudomonas, Clostridium, Micrococcus* and *Bacillus* species from ready to eat barbecue Suya meat in Lagos State. Ananias and Roland (2016) [6] also reported the presence of different bacterial strains from different meat types from highway market in Uganda.

D. Bacterial Contamination Based on Meat Type

Analyzing meat type singly showed that BC were more contaminated with G –ve rods and cocci with a % rate of 69.2 % and 15.4 % compared to G+ve rods and cocci with 7.7 %. *Proteus Sp* was the most common contaminant**(Figure 2).***Proteus* is responsible in spoilage of raw meat, it is also an indication of unhygienic meat preparation. Recent report by Gong *et al* (2019)[7] report *Proteus* as putative gastrointestinal pathogen with high implication in foodborne infection.Hend and Basma (2019)[8]; *Ogbu et al.,* (2016) [9] among other researchers found that roasted chicken sold in Tripoli, Jos and its environs were contaminated with *Salmonella, E.coli, and Shigella, Clostridium, Streptococcus, Klebsiella, Corynebacterium, Staphylococcus, Bacillus and Serratia* species.

Percentage level of Suya meat contamination were 45.5 and 27.3 % for G –ve and G +ve rods compare to 18.2 and 9.1 % for G +ve and –ve cocci with *B. cereus* being the most prominent(**Figure 3).**This is in agreement with the study of Konne *et al..,* (2018)[10] who isolated *B. cereus* as the highest 34 % contaminant of Suya meat sold at Bonny L.G.A of River State.Olatunbosun *et al.,* (2017)[11] also recovered *Bacillus Sp* among other pathogensfrom barbecue Suya meat sold at selected locations in Abuja. Syne *et al.,* (2013)[12] has a documented on microbial hazard associated with ready to eat meat in Trinidad.

Figure 4similar results as that of Suya meat was recorded for FSB though with higher occurrence, S. *aureus* was the leading isolate.*Staphylococcus* is implicated in about 241000 illness of Food borne disease in USA [13] and about 12.5 % of Foodborne outbreak in China. It is said to be the third causative agent of Food borne infection after *Vibrio parahaemolyticus* and *Salmonella Sp*[14].Shi *et al.,* (2018)[15] report 35 % of *S. aureus* from retail meat and meat product in China.

FSB was more contaminated to Suya meat and BC (Figure 5). This may be because there was no application of heat that might have eliminated some microbes.

3. E. Sum Total of Gram Reaction in all Meat Type

Gram (G-ve) rods were more common in all experimented meat types followed by G+ve rods as compared to G -ve and +ve cocci **(Figure 6).** G-ve organisms releases virulence endotoxins and infect substances including meat. These toxins are able to withstand certain degree of heat, thus their survival on roasted meat. Immune reaction of host to these toxins can lead to anaphylactic shock and sometime death of it victims.

3.F.Sum Total of Contamination in All Locations

Bogobiri was more contaminated (44 %) followed by Ekpo Abasi (25 %) compared to Abang Asang, yellow Duke with 16 % and 15 % level of meat contamination. Population is the major factor when considering location in experiment.The aforementioned are locations with high business activity attracting more people toward there more to the later which is just a passer-by- street roads.

G. Antimicrobial Activity of Selected Chemotherapies

The antibiotic sensitivity of tested bacterial isolates showed different susceptibilities ranging from sensitive, intermediate and resistant against different tested antibiotics as indicated in the table 1.

IV. CONCLUSION.

Microorganisms are ubiquitous, with some thermophilic characteristics, thus, their ability to survive everywhere including high heat cannot be undermined. The study reveals the association of pathogenic organisms on freshly slides cow beef, ready to eat Suya and barbecue chicken. We are making and urgent call on National Agency for Food and Drug Administration (NAFDAC), Food Safety Agency etc to reinforce a more formidable team for regular check on road side **ready to eat food**

before allowing consumers access. This and other control measures will minimize regular food borne outbreaks.

V. Declaration

A. Not applicable

B. Consent for Publication

No details, images or videos relating to any individual were used in this research. Therefore, there is no need for "consent for publication".

C. Availability of Data and Materials

All relevant raw data and information given in this manuscript should be freely available to any scientist wishing to use them for non-commercial purposes. It does not breach the participant confidentiality whatsoever.

D. Competing Interests

The authors declares that they have no competing interests.

E. Funding

This research work was fully funded by the authors.

F. Authors' Contributions

The authors have made substantial contributions to the conception, design, analysis, field work, drafting and editing of the work. The authors have also agreed to be personally accountable for the work.

Acknowledgment

Sincere appreciation goes to the Street vendors that serve as our source of sample collection. Special thanks to the laboratory staff and technologists of the Department of Microbiology, Cross River State University (UNICROSS), for their technical assistance and contributions to the success of this work.

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